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EFFECTS OF THREE EMULSIFYING AGENTS AGAINST CRUDE OIL ON THE PRIMARY PRODUCTIVITY OF AN EXPERIMENTAL COMMUNITY OF BENTHIC DIATOMS

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Environmental Protection Agency
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[Effets de trois agents émulsionnants anti-pétrole sur la productivité primaire dune communauté expérimentale de diatomées benthiques]

J. C. Lacaze

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EFFECTS OF THREE EMULSIFYING AGENTS AGAINST CRUDE OIL ON THE PRIMARY PRODUCTIVITY OF AN EXPERIMENTAL COMMUNITY OF BENTHIC DIATOMS

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SUMMARY

Study of modifications in the taxinomic composition and the primary production of a micro-community of benthic diatoms following an acute pollution by various anti-oil emulsifying agents. This microcommunity is settled on supports of polished glass, immersed in a brackish ecosystem in the laboratory. One of the reagents, the Gamasol, used extensively at the time of the shipwreck of the "Torrey-Canyon" is shown to be particularly dangerous since a 30 minutes-exposure to a concentration of 40 mg/l destroys completely the community.

I. INTRODUCTION

The pollution of sea waters by hydrocarbons represents a chronic danger of hydrobiological unbalance prejudicial to the economy of the ocean and lagoon environments. To face this threat, many anti-oil reagents have been devised. Some, like the emulsifying agents poured extensively at the time of the "black tide" caused by the tanker "Torrey Canyon" (March 18, 1967), have been shown to be particularly toxic to the marine flora and fauna (Smith 1968).

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* Technical collaboration H. Herbulot-Sapin

These reagents must then be studied more deeply before any important use in the natural environment.

In this work, we compare the effects of three emulsifying agents upon the photosynthetic activity of a benthic marine community, formed almost exclusively of diatoms. This type of community of microphytes clinging to solid immersed substrates is called a periphyton. This appears as an interesting biological material to study water pollution. In fact, if the anti-oil reagents are used most often to eliminate the floating oil-spills and can then affect the planctonic community, they are also poured, sometimes in large amounts, on the shores where their action on the periphyton must be considered with attention since it has a non-negligible role on the economy of the waters.

The community under study is an experimental community developed on artificial supports immersed in a brackish ecosystem in the laboratory. This type of simplified laboratory microcommunity can easily be subjected to experimentation, and has been studied in detail , in particular by the Laboratory of Botanics of the University of Corvallis (U.S.A.): effects of variation of temperature (Phinney and McIntyre, 1965), effects of illumination and dessication (McIntyre and Wulff, 1969),on the speed of the streams (McIntyre,1966).

II. MATERIALS AND METHODS

The brackish laboratory ecosystem in this study was built following a protocol described previously (Lacaze, et al, 1969); and summarized here: water, algae and sediments with their associated organisms are harvested in a brackish pond in the area around Arcachon, at Station p 229 (in Amanieu, 1967), then brought to the laboratory of the Institute of Oceanography, in Paris (July, 1969). The water is poured in a large polyvinylchloride tank, containing 3,000 liters: 3 kg each of the green algae Chaetomorpha Lineum Kutz and Cladophora Parriaudii Van der Hock were set up on a tray formed of a rigid polyvinylchloride frame covered by a nylon net. The sediments are homogenized and set at the bottom of the tank, about 1 cm thick, (Figure 1A).

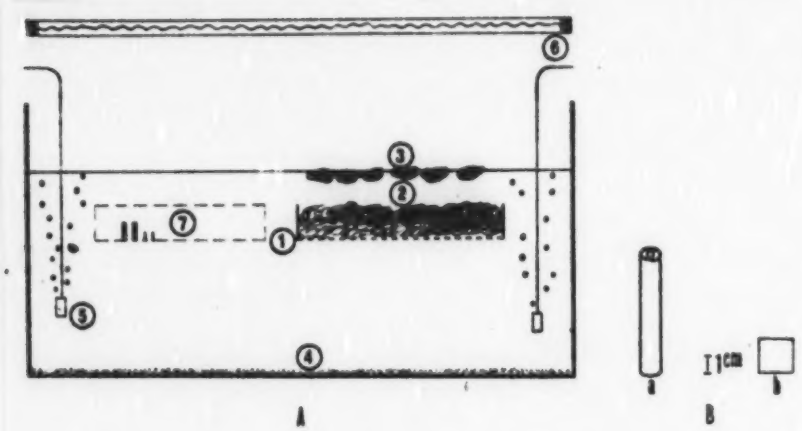

Figure 1.

A- Diagram of an experimental tank. 1- tray for algae; 2- chaetomorpha; 3- cladophora; 4- sediments; 5- diffuser; 6- fluorescent light; 7- place for the support;

B- Support of polished glass. a- test-tube; b- cover-slip.

These ecosystems being established in a laboratory, it is possible to control certain parameters usually variable in the natural environment: temperature (19° C), illumination (3000 lx, Sylvania gro-lux tubes), photoperiod (12:12), chlore content (13.5 g/l).

1. Experimental apparatus

The photosynthetic activity is measured by two different methods: carbon 14, and pH-carbonic gas. A different type of support is used for each method (Figure 1 B). For the ^{14}C method, we used polished glass coverslips (20x20 mm), set up in slits made in Rhodorsil tubes; these tubes maintain the coverslips in a vertical position in the ecosystem, at a depth of 10 cm, and at a distance of 1 cm from one to the other. The less sensitive method of pH-carbonic gas requires a larger surface of periphyton. We have used thick polished glass tubes, of 32 cm^2, suspended at the same depth as the coverslips by nylon strings.

TABLE I *

Relative abundance of various diatoms of the periphyton on artificial polished glass supports, 3 months after immersion in the ecosystem (C: common species; CC: species very abundant). The periphyton contains also many non-identified Nitzschia, Synedra and Amphiprora.

C *Achnanthes brevipes* Ag.	*M. granulata* Grun. Cl.
C *A. brevipes* var. *intermedia* (Kütz) Cl.	*M. pusilla* Grun.
A. hauckiana Grun.	*M. pusilla* var. *breviseta* Cleve-Euler
C *A. longipes* Ag.	*M. tenuissima* Voigt
A. orientalis Hust.	*Navicula accomoda* W. Sm.
Actinoptychus adriaticus Grun.	*Navicula directa* W. Sm. ex Ralfs
A. splendens (Shadb.) Ralfs	*N. gregaria* var. *subtilis* Greg. ex Cl.
A. undulatus Ehr.	*N. halophila* var. *subcapitata* Hust.
C *Amphiprora denticulata* Grun.	*N. membranacea* f. *minuta* Hilse ex Grun.
A. paludosa var. *hyalina* Donk.	*N. microbrebis* Brèb. ex W. Sm.
C *Amphora angusta* Greg.	*N. peregrina* Ehr. ex Kütz.
A. exigua Greg.	*N. pusilla* W. Sm.
C *A. hyalina* Kütz.	*Navicula subminuscula* (W. Sm.) Grun.
A. proteus Greg.	*N. subtilissima* W. Sm.
C *A. ventricosa* Greg.	*N. cf. communissima* Grun.
Auliscus caelatus var. *simplicissima* A. Schmidt	*N. cf. elongata* Grun.
C *Caloneis westii* (W. Sm.) Hendey	*N. jamaicensis* Grun.
Campylodiscus clypeiformis Grun.	*N. liratiformis* (Grun.) subtilis Grun.
C. impressus Grun.	C *N. salinicarum* Brèb. ex Grun.
Ceratoneis amphioxys Ralfs	*N. punctata* W. Sm.
Cocconeis pediculus Ehr.	*N. punctata* var. *coarctata* Grun.
C. pellucida Hantzsch	*N. cf. rostrata* Grun.
C. placentula var. *euglypta* (Ehr.) Grun.	*N. salinarum* D. & Grun.
C. scutellum Ehr.	C *N. subtilissima* Grun.
C *C. scutellum* var. *parva* Grun.	*Pleurosigma elongatum* (Greg.) Harv.
Coscinodiscus centralis Ehr.	C *Pleurosigma elongatum* W. Sm.
C. concinnus W. Sm.	*Rhoicosphenia marginata* (Kütz.) O.M.
C. excentricus Ehr.	*R. marginata* var. *gibberula* Kütz.
Diploneis bombus A. Schmidt	C *Synedra amphicephala* Ag.
D. bombus Ehr.	*Synedra famelica* Ehr.
D. didyma Ehr.	*S. gaillonii* Ehr.
D. mediterranea (Grun.) Cl.	*S. gaillonii* var. *lanceolata* (W. Sm.) V.H.
Gomphonema obtusatum (Lyngb.) Kütz.	*S. ulna* (Nitz.) Ehr.
Hyalodiscus radiatus Bail.	*Synedra* sp.
H. subtilis Bail.	*Synedra tabulata* Grun.
CC *Mastogloia angulata* Lew.	C *S. tabulata* var. *fasciculata* Grun.
M. braunii Grun.	*Trigonium acutangulum* var. *obtusatum* Grun.
M. lanceolata Thw.	*Triceratium formosum* var. *quinquelobatum* Grev.

* Translator's note: Illegible in foreign text.

Four months after the formation of the ecosystem, the artificial polished glass supports are immersed for a period of 3 months.

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After this period of time, some of the supports are taken out for an analysis of the Diatoms-microflora (Table I), of the pigments, and of their products of degradation. The leftover supports are divided into four equal parts, each being immersed in a 4 liter-beaker containing 2 liters of the ecosystem water. A control beaker does not get any polluting substance. Each of the 3 other beakers receive 40 mg/l of one of the 3 anti-oil "detergents". The exposure periods are varied (1, 15, 30 minutes; 3 and 10 hours). A first measure of the phosynthetic activity is performed immediately after the exposure to the reagent (short term effects), then on days 5, 10 and 30 (long term effects). The photosynthetic activity is measured simultaneously by both methods: ^{14}C and pH-CO_2 in the first series. Later, we only used the ^{14}C method which is faster for bio-assays.

2. ^{14}C Method

The ^{14}C method, developed originally by Steeman Nielsen (1952) to measure the primary production at sea, is adapted here to the measure of productivity of the experimental periphyton. The small surface of the glass coverslips allows a direct count of the radioactivity*. After exposure to the polluting substances as indicated above, the coverslips are set into polished-glass 100 ml flasks, with a wide mouth, and 3 µc of $NaH^{14}CO_3$ is injected. The flask contains water from the ecosystem previously filtered thru an HA millipore (0.45 ± 0.02 µ), and one coverslips is set in each flask. The periphyton is cleaned off one side of the coverslips and this side will be applied against the bottom of the flask during the incubation. The flasks are closed, then submersed in the tank at a depth of 10 cm, in the position initially occupied by the supports. For this method, and for the pH carbonic gas method, the incubations are performed during the first five hours of the illumination of the photoperiod, i.e., during the maximal productivity period: in those experimental systems without "dusk", the maximal productivity is reached at the beginning of the

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* Dr. J. Brouardel suggested this method and has kindly helped us with the problems concerning the primary productivity of the ^{14}C method.

"day" (Beyers, 1963). After incubation, the coverslips are dried up in a dessicator with silicagel. A geiger counter with a thin terminal-window, equipped with T GC2 tubes "Tracerlab Waltham" and with impulses-counter Saphymo, Paris, is used for counting the periphyton-side of the coverslip. The values are obtained by the "black" flasks in parallel with "clear" flasks.

The incorporation of ^{14}C on the previously polluted coverslips is compared to the activity in the controls, and the results expressed as percentages.

3. pH-carbonic gas method

A ratio is established between the pH and the total CO_2. Changes in the CO_2 concentration are measured by this ratio CO_2/pH. (Beyers and Odum, 1959, 1960; Beyers, et al., 1963; Beyers, 1964). Once this ratio is known, the slightest variation in pH can express the variation in total CO_2. Beyers (1963 a and b, 1964) measured the metabolism of experimental ecosystem-communities in laboratory by the pH-CO_2 method. The pH is recorded and a curve of the relative variations of CO_2 established, giving a reading of the metabolic activity of the ecosystem for any given hour of the day or night.

We did not actually record the pH; the pH is simply measured at the beginning and at the end of the period of incubation of the supports. The chosen experimental protocol is very similar to the one used for the ^{14}C method, except for the fact that in the pH-CO_2 method, the coverslips are replaced by test tubes. A series of "black flasks" (covered by aluminum foil) is in parallel with the "clear flasks". The respiratory activity obtained is then added to the value of the net photosynthesis given by the "clear flasks".

The variations in pH allow us to measure the impact of pollution on the photosynthetic activity of this type of community. After millipore filtration (HA type), the water of the ecosystem used in the incubation of the cylinders is bubbled through nitrogen. The CO_2 is flushed and the pH increases. The bubbling is stopped when the pH of the sample is above the extreme value of the experiment. The water is titered with CO_2 - saturated

distilled water (bubbling 20 minutes) under a vacuum of 1 atmosphere with a tonometer-burette. A titration with gas-CO_2 is then performed using distilled water for support. For the calculations, the constant change in volume brought about by the change in the amount of water of the sample to titer after each addition of the titrating reagent, must be corrected for, on the basis of 1 liter, using a correction factor. The concentration in CO_2 of the titrating liquid is also a function of the atmospheric pressure and of the temperature, and another correction factor must be introduced. These two factors are in the table (Beyers, et al., 1963). The product of those two factors for a given pH indicates the corresponding CO_2 concentration in millimoles per liter for the experimental water. The product of the factors can be plotted on a curve against the pH values.

Figure 2 presents one of the titration curves of the incubation water. Five other curves were plotted, and all were superposable to the first one. The pH-CO_2 method also gave us the value of the total daily productivity of the periphyton (239 $mg/cm^{-2}/day^{-1}$). Three successive incubations, 4 hours each, were done in order to cover the whole "day" of the ecosystem (12 hours). The total consumption of CO_2 for 12 hours can then be expressed into production of carbon per m^2 and per day.

4. Composition of the 3 anti-oil reagents

The Camosol * was poured in large amounts at the time of the shipwreck of the oil-tanker "Torrey-Canyon" (18 March 1967), releasing to the British Channel 120,000 t of crude oil coming from the oil fields of Kuwait. Very large quantities of this emulsifying agent were used to clean up the French beaches. This component of average density 0.92 is a mixture of non-ionic surfactants and aromatic solvents (Anonymous 1967, Gillerot and Caron, 1967). The surfactants come from the same initial hydrophobic material: the nonylphenols

* Gamlen and Naintre Co.

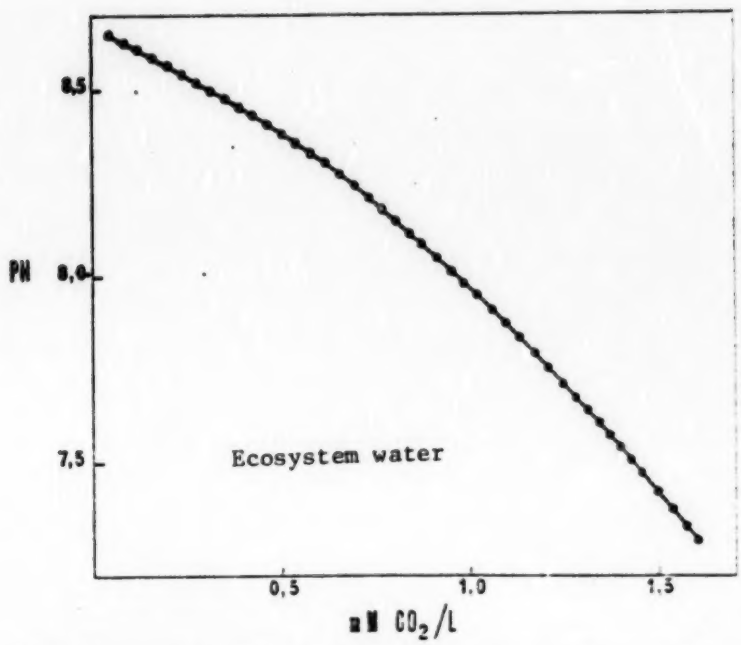

Figure 2. Titration curve of the ecosystem water. From this curve a variation in the ecosystem-water-CO_2 can be deducted from a variation of pH.

commercial name for a mixture of monoalkylphenol isomers), but different by the amount of hydrophilic components (ethylene oxide). By raising the amounts of the latter, various ratio hydrophilic/hydrophobic are obtained. The number of ethylene molecules varies between 4 and 9. The surfactants represent only 14% of the total mixture; the solvents represent 86%: those are aromatic hydrocarbon (substituted benzene) residues of kerosene distillation. The Sefoil * of density 1.56, is formed of carbon hydrates and phosphoric acid (Fusey, et al., 1969). For use, this product is a mixture of 100 parts of hydrocarbons to 60-70 parts of Sefoil until an emulsion is produced. The Corexit 7664, density 1,025, is described as biodegradable and nontoxic ** . The amount to use in the treatment and removal of oil spills varies with the nature of the hydrocarbons spilled and the water gemperature. It is between 1 and 10% the volume of the spill.

* Dambros Society

** Technical Bulletin, Esso.

III. RESULTS AND DISCUSSION

1. Taxinomic composition and primary production of periphyton.

After three months of immersion in the ecosystem, the test tubes and coverslips are covered by a microcommunity of diatoms (Table I) of the main groups hereafter: Achnanthes brevipes Ag., A. brevipes var intermedia (Ktz), Cl., A. longipes Ag., Amphiprora decussata Grun., Amphora angusta Greg., A. hyalina Ktz., A. ventricosa Greg., Caloneis vestii (W. Sm.) Hendey, Cocconeis scutellum Ehr., Mastogloia angulata Lew. Nitzschia navicularis (Breb.) Grun., N. subcalcareus Grun., Pleurosigma elongatum W. Sm., Striatella unipunctata Ag., Synetra laevigata var. hyalina Grun. A species new in science, Mastogloia lacazei, also abundant in this environment, was described by Voigt (1970). The Cyanophycae Spirulina subsalsa Oerst., Oscillatoria chalybea Mert., Lyngbya lutea Gom., the Chlorophycae Ulvella sp. and Dinoflagellatae Exuviaella sp. are also present, but in small number.

The daily analysis of the community does not indicate any settlement of diatoms for the first 15 days; later on, from the 15th to the 30th day, the following species are getting established:

Achnanthes hauckiana Grun.
A. orientalis Hust.
A. longipes Ag.
Amphiprora paludosa var. hyalina Donk.
Amphora hyalina Ktz.
A. exigua Greg.
A. ventricosa Greg.
Cocconeis scutellum Ehr.
C. scutellum var. parva Grun.
Navicula peregrina (Ehr.) Ktz.
Nitzschia incurva Grun.
N. subcalcareus Grun.
N. lorenziana v. subtilis Grun.
Rhopalodia musculus (Ktz.) O.M.
Striatella unipunctata Ag.
Synedra laevigata Grun.

From the 30th to the 40th day these species form colonies. Two species are also added: Pleurosigma elongatum W. Sm. and Mastogloia angulata Lew. From the 40th day on, each species is represented by individual colonies.

The formation of real colonies starts by small groups of Monoraphides diatoms. These first colonies act as a support for heavier forms and for the diatoms fixed by stalks and peduncles, and it is on this carpet that we find later the Mastogloia, Pleurosigma and other Naviculacae.

The heterogeneity of the periphyton population is a major hindrance to a quantitative study of this material (Wetzel, 1965). On the immersed supports, the abundance of harpaticoides copepods at all stages of development (Ameira parvula (Claus.), Heterolaophonte stromi (Baird), Harpacticus littoralis Sars could also alter the homogeneity of the periphyton. In fact, the results of chlorophyll-a determinations (0.367 ± 0.080 $\mu g/cm^2$)* and of phaeo-pigments (0.164 ± 0.016 $\mu g/cm^2$), calculated by the Lorenzen (1967) Formula for the analysis of each 7 test tubes, indicate a fair homogeneity of the algae on the experimental supports.

In preliminary experiments on brackish ecosystems of the same type, and with a similar experimental protocol (Lacaze, et al., 1969), the analysis of microscope slides immersed at the beginning of the experiment showed a periphyton of the same taxinomic composition. This similarity can be explained: the harvesting of the natural environment was done at the same place and time (July). It must be remarked upon also that the taxinomic composition of the community seems to be the same on the polished test tubes and on microscope slides, and can be compared to the one described for another laboratory experimental ecosystem by McIntyre and Wulfe (1969), who studied the effects of illumination and drought on the settlement of diatoms. Some species are common to both communities (Achnanthes brevipes Ag., A. brevipes var. intermedia (Ktz), Cl., A. haukana Grun., A. Longipes Ag., Cocconeis scutellum Ehr., C. scutellum var parva Grun., Coscinodiscus exentricus Ehr., Navicula directa (W. Sm) Ralfs), though the experimental conditions were completely different. The ecosystem described by these two authors gets water continuously from the Pacific Coast of the United States, when ours, in the opposite, functions in closed circuit and contains also some sediment and 6 kg of macro-algae which probably compete with the diatoms and slow down their development, and therefore explains the low primary productivity of this community

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* Standard deviation.

(239 mg/Cm^{-2}/day^{-1}) as compared to other experimental periphyton colonies (McIntyre, et al., 1964; Prinny and McIntyre, 1965; McIntyre and Wulff, 1969).

2. Effects of three emulsifying agents

Very different effects are observed for the anti-oil reagents studied. Two of these substances, the Corexit and Sefoil, do not alter the photosynthesis at the concentration of the experiment (40 mg/l^{-1}); but the Gamosol has an obvious effect. A brief exposure (1 to 15 minutes) of the community to the Gamosol shows a definite stimulation (Figure 3). An exposure of 30 minutes, on the opposite, gives a slight decrease; after 10 hours the photosynthetic activity of the community having undergone pollution is only 30% of the value of the controls. The two methods for the measurement of photosynthesis, used simultaneously in this first series,give very similar results.

The analysis performed immediately after exposure to the Gamosol is enough to determine its toxicity to the biocoenoses. Thus, a 30-minute exposure to the Gamosol which at first induces only a slight decrease of photosynthesis, stops it completely within 5 days.

This total absence of photosynthesis, also obvious for longer exposures (3 to 10 hrs.),indicates a destruction of the majority of the species of the biocoenoses. The disappearance is,however, only temporary; then comes a progressive recovery so that after 20 days the photosynthesis will be the same as the controls; and doubled after 30 days. The fixation of ^{14}C will be finally more important on the cover-slips which have been exposed to longer times of pollutant (Figure 3). These results lead us to a sharp distinction between the short-term effects, observed immediately, and the long-term effects.

On the other hand, microscopic examinations one month after the beginning of the experiment show that the cover-slips exposed for 30 minutes, 3 hrs. and 10 hrs. to the Gamosol are almost exclusively covered by the diatom

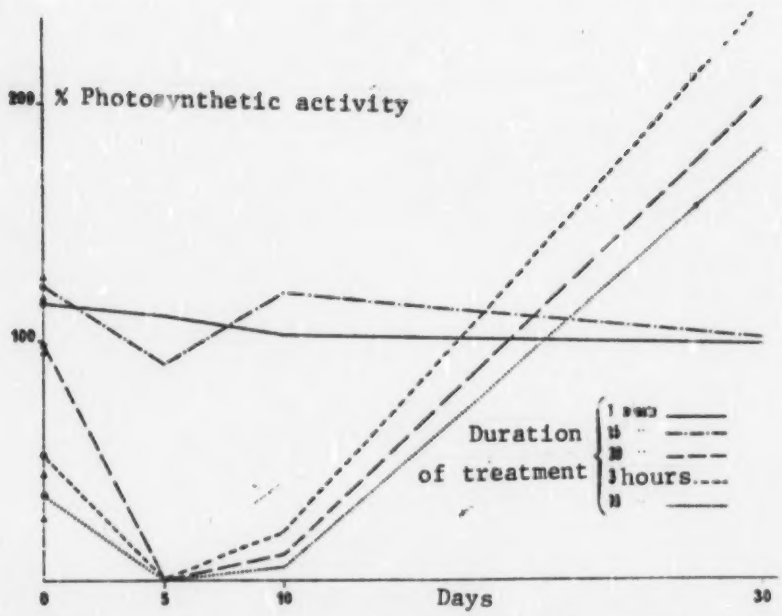

Figure 3. Effects of Gamosol on the photosynthetic activity of the periphyton, in percentage of the controls. ^{14}C method (o), pH-CO_2 method (Δ).

Coconeis placentula var. euglypta (Ehr.) Grun, which settles on the empty crusts left by the community destroyed by the pollution. Other small size diatoms are also present: Amphora sp., Navicula sp., Nitzschia sp.

The effects of pollution are then distinctively expressed by a simplification of the community structure, a decrease of the number of species, each being extremely populated. We have observed that this change in structure is concommitant to an increase of the photosynthetic activity of the community. Such disturbances, even if they correspond to an increase in productivity, are not necessarily beneficial to the natural environment; a diversified periphyton, even weakly productive, might be preferable for some food-chains of economic interest. On the other hand, it is not necessary for the pollution to be tremendous to reach some stages of the vital cycle of economically important organisms; an indirect action on their alimentary element can cause an important damage.

3. Dangers of the Emulsifying Agents Type "Torrey-Canyon".

The results of the experiments with Gamosol indicate a strong toxicity destroying the biocoenose after a 30-min., 40 mg/l^{-1} exposure. These recults are similar to the ones obtained with axenic cultures of the alga Phaeodactylum tricornutum (Lacaze 1969): they are also destroyed by a 30-min. exposure to Gamosol. The solvent fraction of the Gamosol might be responsible for this algicide effect, since the compounds of the benzene series included are particularly toxic (Korzep 1962). This toxicity is fortunately diminished by the fact that the solvent is volatile in diluted solutions (Lacaze 1967). At the Plymouth Laboratory (Smith 1969), experiments have demonstrated that after 100 hours, the solvent fraction of a 10 ppm solution of an anti-oil emulsion such as Gamosol is completely eliminated. In our case, the short exposures allow us to think that the loss of solvent is negligible.

It would be premature, however, to conclude that the surfactants are nontoxic. If they do not seem to be algicide at the concentrations used in our study, they slow down or inhibit the development of some cultures (Ukeles 1965, Lacaze 1967, 1969) and of natural bioceonoses at a few mg per liter concentration. On the other hand, we have demonstrated the slow biodegradability of these surfactants in the natural environment (Lacaze 1973). This disadvantage is not found in Sefoil, which favors the petroleum degradation (Fusey, et al., 1969).

This study demonstrates the necessity of long-range experiments which would make a distinction between an <u>algistatic</u> and an <u>algicide</u> action; and indicates that variation of the taxinomic composition and of the primary production of experimental bioceonoses are a sensitive assay for the impact of pollution on water resources. The search for the effects of a pollutant on the biomass settled in a laboratory microecosystem is an important development, which must be completed by toxicologic studies on representative samples of isolated species (Fontaine 1969). The very sensitive ^{14}C method allows us to detect even minimal variations in the metabolism.

The periphyton, representing an important element of the biota, and one of the bioceonoses threatened most often by shores pollution, appear to be an excellent study material.

SUMMARY

The effects of three anti-oil emulsifying agents on the taxinomic composition of the primary productivity of an experimental community of benthic diatoms are studied. This association of micro-algae develops on artificial polished glass-supports immersed in a 3000 liter brackish ecosystem, set up in the laboratory. The community's photosynthetic activity is measured by two different methods: carbon 14 and pH-carbonic gas. The exposure periods are varied (1, 15, 30 minutes, 3 and 10 hours) for the concentration of 40 mg/l^{-1} of each of the 3 anti-oil substances. The effects of the pollution are studied immediately after exposure to the substances (short-term effects), then after 5, 10, 30 days (long-term effects).

Two of the anti-oil substances (Sefoil and Corexit) do not change primary production and therefore, in this aspect, show no toxicity under test conditions. On the other hand, Gamosol, a substance used massively when the tanker "Torrey Canyon" was shipwrecked (18 March 1967) is distinctly toxic.

This research involves methodological problems. Only long-term tests make it possible to show the real effect of a polluting substance. Thus, a 30-minute Gamosol exposure which at first induces only a slight decrease of primary production after 5 days puts a complete end to it. Microscopic observation shows that the algae forming the community are destroyed through this exposure. A new community made up of small diatom species then develops rapidly so that after 30 days, primary production of the communities having undergone pollution is twice that of the control community..

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